

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1.-11. (Cancelled)

12. (Previously presented) A method for recombinase mediated expression cassette exchange (RMCE) for substituting a positive-negative selectable marker by an incoming DNA in the genome of cells or parts of cells comprising the steps of

- a) integrating into a chromosomal locus of the genome of said cells a first DNA expression cassette carrying a positive-negative selection marker flanked by a wild type FLP-recombinase recognition target (FRT) site on one end and a modified heterospecific FRT site on the other end, wherein said modified heterospecific FRT site is an F₃ spacer mutant,
- b) selecting cell clones surviving the conditions for positive selection,
- c) exchanging said first DNA expression cassette against an incoming second DNA expression cassette located on a circular vector and carrying a homologous or heterologous gene (transgene) of any coding sequence flanked by the same FRT sites as said first DNA expression cassette mediated by the action of FLP-recombinase, wherein said cells are vertebrate embryonic stem cells (ES) and said parts of cells are nuclei of vertebrate cells, which can be inserted into ES cells, and wherein
- d) maintaining the conditions for positive selection during cultivation of said cells obtained in step b) for a time sufficient to exchange said first DNA expression cassette against said incoming second DNA expression cassette,
- e) using in step c) an incoming second DNA expression cassette which is marker-free, and

- f) selecting cell clones obtained after step c) surviving the conditions for negative selection,
- g) wherein said positive and negative selection conditions result in recombination frequencies of at least 25%.

13. (Previously Presented) The method of claim 12, wherein said positive-negative selection marker is a hygromycin-B-phosphotransferase and HSV-thymidine kinase encoding (hyg^{tk}) fusion gene.

14. (Previously Presented) The method of claim 1, wherein said vertebrate embryonic stem cells are mouse embryonic stem cells.

15.-17. (Canceled)

18. (Currently Amended) A method for generation of a chimera transgenic mice comprising the step of injecting mouse ES cells comprising a modified genome obtained by the method of claim 12 into the blastocysts of a mouse ~~said mice~~.